Functional characterization of grapevine MLO genes to define their roles in Powdery mildew susceptibility by CRISPR/Cas9 genome editing

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Abstract

Successful powdery mildew (PM) infection in plants relies on *Mildew Resistance Locus O* (*MLO*) genes, which encode susceptibility factors essential for fungal penetration. In *Arabidopsis*, loss-of-function mutations in three clade-V MLOs, *AtMLO2*, *6*, and *12* confer complete resistance to PM infection. Since then, efforts are on to discover MLO genes contributing to PM susceptibility in many species to introduce *mlo*-based PM-resistance. Earlier studies in tomato and grapevine, using the RNAi approach, attributed PM susceptibility to *SlMLO1*, *5*, and *8* and *VvMLO3*, *13*, and *17*, respectively indicating likely functional redundancy among MLOs. Here, we disrupted the closest grapevine orthologues, *VvMLO3*, *4*, *13*, and *17* through CRISPR/Cas9-mediated mutagenesis in the microvine model with the goal of identifying the candidate MLO genes to introduce *mlo*-based PM resistance. Individual mutants *mlo3*, *mlo4*, *mlo13* and *mlo17* showed 8 to 50% less infection to *E. necator*, whereas double mutants, *mlo3/4*, *mlo3/13* and *mlo13/17* and triple mutant *mlo3/13/17* showed 60 to 90% less infection. But the quadruple *mlo3/4/13/17* mutant plants showed near complete PM resistance. Considerable differences were observed in the resistance level of clones among the triple and quadruple mutants due to the differences in editing efficiency of individual guide RNAs. Some mutants showed pleiotropic effects in the growth and development, ranging from early senescence and stunted growth to non-flowering phenotypes, which also seemed to depend on the percentage of gene-edited cells in the plant. The overarching goal is to excise the genome-integrated T-DNA cassette from the mutants using CRISPR Ribonucleoproteins for transgene-free PM resistance.

Keywords: Powdery mildew, Grapevine MLO, mildew-resistance, Gene Editing.